CONFIRMATION OF CANNABINOIDS BY LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

27.1 POLICY

This test method may be used to confirm the presence of Δ^9-THC (THC) and its metabolite, 11-nor-9-carboxy-Δ^9-THC (THCCOOH) in biological samples and other submitted evidence. Quantitative results obtained through the use of this method will only be reported within the validated dynamic range. Reporting of results following the application of this method will be contingent upon a thorough review and acceptance of quality control data and the qualification of individual results under the criteria for acceptance.

Any adjustments or deviations from the procedures below must be approved by a member of TLD Management, and appropriately documented in the batch file.

27.2 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and quantitation of THC and THCCOOH present in biological specimens and other submitted evidence. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis and criteria for acceptance for batch data from method validation.

27.3 PRINCIPLE

The targeted compounds and internal standards are isolated from whole blood, serum, plasma, urine and other biological samples or evidence by the use of liquid-liquid extraction (LLE). Following LLE, the specimens, now termed extracts, are injected into a high performance liquid chromatograph (HPLC) where they are separated between a liquid mobile and liquid stationary phase. Each compound exits the HPLC at a reproducible time which is termed its retention time.

The HPLC is coupled to a tandem mass spectrometer (MS-MS) detector equipped with an atmospheric pressure electrospray ionization source. As each ionized compound is drawn into the high vacuum region of the instrument, selected-ion and multiple-reaction monitoring is used to measure the mass-to-charge ratios of each compound and its related fragments. Multiple-point, internal standard calibration is used to generate a calibration curve. The concentration of any THC or THCCOOH identified in a sample is determined from its calibration curve.

27.4 SPECIMENS

27.4.1 The specimen/sample volume is 1 mL for all specimen types.

27.4.2 Specimens/samples include whole blood, serum, plasma, urine, tissue homogenate and non-biological aqueous solutions or solid material.

27.4.3 Dilutions of specimens/samples may be analyzed at the Forensic Scientist's discretion; in addition, the specimen may be analyzed at standard volume, as dictated by screening results, to ensure that concentrations of all target compounds present are within the dynamic range of the test method.

27.4.4 Analysis of larger specimen/sample volumes must be approved and documented.

27.5 REAGENTS, MATERIALS AND EQUIPMENT

27.5.1 REAGENTS

27.5.1.1 Acetic acid, glacial

27.5.1.2 10% Acetic acid

Add 10 mL of concentrated acetic acid to 80 mL DI H_2O in a 100 mL flask. Dilute to 100 mL with DI H_2O and mix. The solution is stored in a glass bottle at room temperature and expires one year from the date of preparation. Adjustments to final volume are permitted as long as the proportions are maintained.

27.5.1.3 Acetonitrile (ACN)

27.5.1.4 Certified blank blood (specific to THC assay)

27.5.1.5 Deionized water (DI H_2O)

27.5.1.6 Ethyl acetate (EtAC)

27.5.1.7 Extraction solvent, hexanes:ethyl acetate 9:1 (for use on date of preparation only)

Add 90 mL hexanes to a glass flask. Add 10 mL ethyl acetate and mix. Adjustments to final volume are permitted as long as proportions are maintained.

27.5.1.8 Formic acid, concentrated

27.5.1.9 0.1% Formic acid

Add 1 mL of concentrated formic acid to 800 mL DI H_2O in a 1 L flask. Dilute to 1 L with DI H_2O and mix. Filter this solution prior to use on the HPLC. The solution is stored in a glass bottle at room temperature and expires one year from the date of preparation. Adjustments to final volume are permitted as long as the proportions are maintained.

27.5.1.10 Hexanes

27.5.1.11 Methanol (MeOH)

27.5.1.12 Reconstitution solution, 50:50 ACN:DI H_2O (for use on date of preparation only)

Add 2 mL of acetonitrile to 2 mL of DI H_2O in a glass tube, cap and mix. Adjustments to final volume are permitted as long as proportions are maintained.

27.5.1.13 Sodium hydroxide (NaOH), concentrated, 10N

27.5.2 MATERIALS

27.5.2.1 Autosampler vials (glass with integrated conical inserts) and caps

27.5.2.2 Disposable 16 x 125 mm tubes with safety closures

27.5.2.3 Disposable screw-cap tubes or centrifuge tubes with safety closures

27.5.2.4 Disposable pipette tips

27.5.2.5 Disposable glass transfer pipettes

27.5.2.6 HPLC Column, Agilent Poroshell 120 EC-C18, 2.1x75 mm, 2.7µM particle size, or equivalent

27.5.2.7 Laboratory glassware (graduated cylinders, flasks)

27.5.2.8 pH indicating paper

27.5.2.9 Volumetric glassware (flasks)

27.5.3 EQUIPMENT

27.5.3.1 Agilent HPLC (1100/1200 series or equivalent)

27.5.3.2 Agilent MS-MS with API-ES source (6410, 6420, or equivalent)

27.5.3.3 Calibrated, adjustable air-displacement pipettes

27.5.3.4 Centrifuge

27.5.3.5 Evaporator (Caliper LS, formerly Zymark, TurboVap)

27.5.3.6 Heating block, oven, dry bath or wet bath

27.5.3.7 Rotary mixer

27.5.3.8 Verified, adjustable repeater-pipette

27.5.3.9 Vortex mixer

27.6 STANDARDS, CALIBRATORS AND CONTROLS

27.6.1 STANDARDS

27.6.1.1 Reference materials (referred to interchangeably in this method as stock standards) are used for the preparation of stock or working standards which, in turn, are used to produce calibrators, positive controls and the working internal standard.

27.6.1.2 Certified reference materials (CRM's) for preparation of working standard and stock internal standard (IS) are purchased from an approved reference material supplier and include the following:

a. Δ^9-THC: 1.0 mg/mL
b. Δ^9-THC-d_3: 0.1 mg/mL
c. 11-nor-9-carboxy-Δ^9-THC: 1.0 mg/mL
d. 11-nor-9-carboxy-Δ^9-THC-d_3: 1.0 mg/mL
e. 11-nor-9-carboxy-Δ^9-THC glucuronide: 0.1 mg/mL

27.6.1.3 Working standard (10, 50 ng/µL)

a. Using calibrated pipettes, measure 500 µL of THC and 2.5 mL of THCCOOH CRM's into a 50 mL class-A volumetric flask.

b. Add MeOH to the flask to the designated volume.
c. The final concentration of the working standard is 10 ng/µL THC and 50 ng/µL THCCOOH. The working standard is stored in the freezer in an amber bottle and expires one year from the date of preparation (if a CRM expires prior to one year from date of preparation, the prepared solution expiration date becomes the first day of the month in which the CRM with the earliest expiration date expires). Volumes may be adjusted provided that proportions remain constant.

27.6.1.4 Stock Internal standard (1, 5 ng/µL)

a. Using a calibrated pipette, measure 250 µL THC-d_3 and 125 µL THCCOOH-d_3 CRM's into a 25 mL class-A volumetric flask.
b. Add MeOH to the flask to the designated volume.
c. The final concentration of the stock internal standard is 1 ng/µL THC-d_3 and 5 ng/µL THCCOOH-d_3. The stock internal standard is stored in the freezer in an amber bottle and expires one year from the date of preparation (if a CRM expires prior to one year from date of preparation, the prepared solution expiration date becomes the first day of the month in which the CRM with the earliest expiration date expires). Volumes may be adjusted provided that proportions remain constant.

27.6.1.5 Working Internal standard (0.1, 0.5 ng/µL)

a. Using a calibrated pipette, measure 2.5 mL stock internal standard into a 25 mL class-A volumetric flask.
b. Add MeOH to the flask to the designated volume.
c. The final concentration of the working internal standard is 0.1 ng/µL THC-d_3 and 0.5 ng/µL THCCOOH-d_3. The working internal standard is stored in the freezer in an amber bottle and expires one year from the date of preparation (if the stock internal standard expires prior to one year from date of working internal standard preparation, the expiration date of the working solution is the expiration date of the stock internal standard). Volumes may be adjusted provided that proportions remain constant.

27.6.2 CALIBRATORS

27.6.2.1 Calibrators are prepared in certified blank blood at the time of analysis using the working standards. The preparation of the calibrators is detailed in 27.7 SAMPLE PREPARATION. If necessary, calibrators may be prepared in alternate matrices provided that the matrix has been previously determined to not contain any of the compounds tested for by this procedure. If the matrix has not been verified as negative, a matrix blank must be included in the batch.

27.6.3 CONTROLS

27.6.3.1 Negative Control

a. At least one negative whole blood control is tested with every batch. The negative control is prepared using certified blank blood.

b. When testing different sample types, wherever possible, include a negative control prepared from that matrix. (For example, when analyzing whole blood and urine samples the batch shall include at least one negative whole blood control and at least one negative urine control.)

27.6.3.2 Positive Controls

a. At least three positive whole blood controls are tested with every batch. The positive controls are prepared using certified blank blood to which the designated volume of control working standard has been added.
b. Control stock standards are obtained from an approved reference material supplier.
c. The control stock standards must be either a different lot number or from a different supplier to those used in producing the working standard. If the same lot must be used, the working control standard must be prepared by someone other than the person that prepared the working standard.
d. The control working standard (10, 50 ng/µL) is prepared as described in 27.6.1.3.
e. The preparation of the positive whole blood controls is detailed in 27.7 SAMPLE PREPARATION. Alternatively, quality control personnel may provide in-house positive controls.
f. When testing different sample types, wherever possible, include at least one positive control prepared from that matrix.

27.7 SAMPLE PREPARATION

27.7.1 Label a clean 16 x 125 mm tube for each member of the test batch. (i.e. calibrator, control, case sample)

27.7.2 Add 2 mL DI H_2O to each tube.

27.7.3 Using a calibrated pipette, add 1 mL of certified blank whole blood into each of the seven calibrator tubes, the positive control tubes and the negative control tube(s).

27.7.4 Prepare a 1:10 dilution of the working standard. (1, 5 ng/µL)

a. Using a calibrated pipette, combine 0.1 mL of the working standard with 0.9 mL of ACN or MeOH in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after calibrator preparation.

27.7.5 Prepare a 1:100 dilution of the working standard. (0.1, 0.5 ng/µL)

a. Using a calibrated pipette, combine 0.1 mL of the 1:10 dilution with 0.9 mL of ACN or MeOH in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after calibrator preparation.

27.7.6 Using a calibrated pipette, spike the calibrators according to the following table, using the dilutions prepared from the working standards.

Calibrator Description (THC/THCCOOH)	Volume (µL) Added	Working Standard
Calibrator 1 (1.0/5.0 ng/mL)	10	0.1/0.5 ng/µL
Calibrator 2 (2.0/10 ng/mL)	20	0.1/0.5 ng/µL
Calibrator 3 (5.0/25 ngm/L)	50	0.1/0.5 ng/µL
Calibrator 4 (10/50 ng/mL)	100	0.1/0.5 ng/µL
Calibrator 5 (25/125 ng/mL)	25	1.0/5.0 ng/µL
Calibrator 6 (50/250 ng/mL)	50	1.0/5.0 ng/µL
Calibrator 7 (100/500 ng/mL)	100	1.0/5.0 ng/µL

27.7.7 Prepare a 1:10 dilution of the control working standard. (1, 5 ng/µL)

a. Using a calibrated pipette, combine 0.1 mL of the control working standard with 0.9 mL of ACN or MeOH in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after control preparation.

27.7.8 Prepare a 1:100 dilution of the control working standard. (0.1, 0.5 ng/µL)

a. Using a calibrated pipette, combine 0.1 mL of the 1:10 dilution with 0.9 mL of ACN or MeOH in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after control preparation.

27.7.9 Using a calibrated pipette, spike the positive controls according to the following table, using the dilutions prepared from the control working standards.

Control Description (THC/THCCOOH)	Volume Added (µL)	Working Control Standard
Control 1 (3.0/15 ng/mL)	30	0.1/0.5 ng/µL
Control 2 (20/100 ng/mL)	20	1.0/5.0 ng/µL
Control 3 (80/400 ng/mL)	80	1.0/5.0 ng/µL

27.7.10 If in-house positive controls are being used, transfer 1 mL of each into their labeled tubes, using a calibrated pipette.

27.7.11 Using a calibrated pipette, sample 1 mL of each case sample into its respective tube.

27.7.12 Using a calibrated pipette or verified repeater-pipette, add 100 µL of the working internal standard solution to each tube and briefly vortex-mix. Final concentration of the internal standard is 10 ng/mL THC-d_3 and 50 ng/mL THCCOOH-d_3.

27.7.13 Add 800 µL of 10% acetic acid and vortex-mix.

27.7.14 Add 8.0 mL of extraction solvent (hexanes:ethyl acetate, 9:1) to each tube.

27.7.15 Cap the tubes and rotate for 30 minutes.

27.7.16 Centrifuge at 2500 rpm for 15 minutes to achieve separation.

27.7.17 Transfer organic layer to appropriately labeled centrifuge or screw cap tubes.

27.7.18 Evaporate samples to dryness at 40°C.

27.7.19 Reconstitute samples with 100 µL of reconstitution solvent (50:50 ACN:DI H_2O) and briefly vortex-mix. If necessary, centrifuge the tubes for 2 minutes at 2000 rpm to collect the extracts at the bottom of the tubes.

27.7.20 Transfer the extracts to labeled glass autosampler vials with integrated conical inserts and cap.

URINE EXTRACTION

Urine specimens require hydrolysis of glucuronide conjugates prior to sample preparation, according to the following procedure:

1. Label a clean 16 x 125 mm tube for each member of the urine test batch.
2. Using a calibrated pipette, sample 1 mL blank urine into each of the two calibrator tubes, the positive glucuronide control tube, and the negative control tube.
3. Using a calibrated pipette, spike urine calibrators at Cal 1 (5.0 ng/mL THCCOOH) and Cal 5 (125 ng/mL THCCOOH) concentrations, using the working standard dilutions, as described in 27.7.6.
4. Prepare a 1:10 dilution (10 ng/µL) of the 0.1 mg/mL THCCOOH glucuronide stock CRM.
   - Using calibrated pipettes, combine 0.1 mL of the CRM with 0.9 mL ACN or MeOH in a labeled tube. Cap and vortex-mix. The dilution shall be disposed of after control preparation.
5. Prepare a 1:10 dilution (1.0 ng/µL) of the 10 ng/µL solution.
   - Using calibrated pipettes, combine 0.1 mL of the 10 ng/µL dilution with 0.9 mL ACN or MeOH in a labeled tube. Cap and vortex-mix. The dilution shall be disposed of after control preparation.
6. Using a calibrated pipette, add 100µL of the prepared 1.0 ng/µL dilution to the glucuronide positive control tube.
7. Using a calibrated pipette, sample 1 mL of each urine case sample into its respective tube.
8. Using a calibrated pipette or verified repeater-pipette, add 100 µL of the working internal standard solution to each tube and briefly vortex-mix. Final concentration of the internal standard is 50 ng/mL THCCOOH-d_3.
9. Add 40 µL of 10N NaOH to each tube.
10. Verify pH is >10 using pH indicator paper.
11. Cap tubes and briefly vortex-mix.
12. Incubate the tubes for 20 minutes at 60°C.
13. Remove from heat and cool to room temperature.
14. Add 25 µL glacial acetic acid to neutralize pH.
15. Briefly vortex-mix.
16. Proceed with sample preparation at 27.7.14.

27.8 INSTRUMENTAL PARAMETERS

The instrumental parameters can be found in Appendix A. Prepare a sequence or batch table by first setting the data path in MassHunter (or data file name in Analyst) to the date of the test. After entering all vial locations and sample descriptions in the worklist/batch table ensure that the method listing in the table is THC (THC.M on Agilent or THC.dam on Sciex) for each line. As needed, the sequence may conclude with an injection that rinses the column and puts the instrument in standby (e.g. using method RINSE.M, THCRINSE.DAM, SHUTDOWN.DAM), or this may be done manually.

27.9 DATA ANALYSIS

27.9.1 Analysis of the batch data is conducted using the instrumental data analysis software in MassHunter (Agilent) or MultiQuant (Sciex).

27.9.2 Quantitative calculations are generated by internal standard, multi-point, linear regression with a $1/a^2$ (inverse of concentration squared) weighting factor. The calibration curves are updated using the calibrator results for the batch; no historical calibration curves are permitted.

27.9.3 For urine confirmation, a two-point calibration curve (equal weighting, origin excluded) is generated, using urine calibrators at Cal 1 and Cal 5 concentrations.

27.9.4 Printed reports for each vial in the batch are generated for review along with the updated calibration curves.

27.9.5 Technical review of the batch is conducted according to the criteria listed below.

27.10 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

27.10.1 Calibrators and calibration curves

27.10.1.1 Chromatographic peaks for THC, THCCOOH and internal standards shall appear symmetrical (i.e. no co-elution, split peaks, or shoulders).

27.10.1.2 Retention times for target compounds and internal standards shall be within ±2% and ion ratios shall be within ±20% of those in calibrator 4. These are inclusive ranges.

27.10.1.3 Quantitative results for THC and THCCOOH in each calibrator shall be within ±20% of their target values with the exception of calibrator 1 which shall be within ±25% of their targets. These are inclusive ranges. For target concentrations <10 ng/mL, result comparisons will use values truncated after the first decimal place in units of ng/mL. For target concentrations ≥10 ng/mL, result comparisons will use whole integer values in units of ng/mL.

27.10.1.4 No calibrators may be removed from the THC calibration curve for the batch to be acceptable for quantitative reporting of THC.

27.10.1.5 The calibration curves for THC and THCCOOH shall have correlation coefficients ≥0.99.

27.10.1.6 The failure to meet any of these criteria for one compound does not invalidate the acceptability of another compound.

27.10.1.7 For urine confirmation, criteria described in 27.10.1.1 – 27.10.1.3.

27.10.2 Controls

27.10.2.1 The negative control(s) shall not identify THC or THCCOOH above its limit of detection. Identification is based on a) acceptable retention

time matching, b) distinct peaks present for all selected ions, and c) acceptable ion ratios. Negative urine control(s) shall not identify THCCOOH above its limit of detection, based on above criteria.

27.10.2.2 Positive controls

a. Chromatographic peaks for THC, THCCOOH and internal standards shall appear symmetrical.

b. Retention times for target compounds and internal standards shall be within ±2% and ion ratios shall be within ±20% of those in calibrator 4. These are inclusive ranges.

c. Quantitative results for THC and THCCOOH in each control shall be within ±20% of their target values. These are inclusive ranges. For target concentrations <10 ng/mL, result comparisons will use values truncated after the first decimal place in units of ng/mL. For target concentrations ≥10 ng/mL, result comparisons will use whole integer values in units of ng/mL.

d. The failure to meet any of these criteria for one compound does not invalidate the acceptability of another compound.

e. All positive controls in the batch must meet acceptability criteria for a target compound in order to report quantitative results for that compound in a case specimen.

f. The positive glucuronide urine (process) control is used to evaluate the effectiveness of the enzymatic hydrolysis. Retention times of THCCOOH and internal standard shall be within ±2% and ion ratios shall be within ±20% of those in the 125 ng/mL THCCOOH urine calibrator. The control is considered acceptable if recovery of THCCOOH is demonstrated, and criteria in 27.10.2.2.a. are met.

27.11 CRITERIA FOR CASE SAMPLE ACCEPTANCE

If the criteria for batch acceptance have been satisfied, the results of individual case samples are deemed suitable for reporting if the following criteria are met.

27.11.1 Any chromatographic peak for THC or THCCOOH and internal standards shall appear symmetrical.

27.11.2 The retention times for THC, THCCOOH and internal standards are within ±2% and the ion ratios are within ±20% of those in calibrator 4. These are inclusive ranges.

27.11.3 The quantitative results for each identified compound must be within the dynamic range of the test method. Results greater than the upper limit of quantitation may be reported qualitatively, provided that all other criteria for acceptance are met.

27.11.4 When dilutions of case samples are tested, the quantitative result(s) before multiplication shall be within the dynamic range of the test method.

27.11.5 Urine samples are suitable for qualitative reporting of THCCOOH if criteria in 27.11.1 is met, the retention times of THCCOOH and internal standard are within ±2% and ion ratios are within ±20% of those in the 125 ng/mL THCCOOH urine calibrator, and the calculated value is ≥ 5.0 ng/mL THCCOOH.

27.12 REPORTING

27.12.1 THC results, and associated measurement uncertainties, are reported to two significant figures, in units of nanograms per milliliter (ng/mL).

27.12.1.1 The full THC result from the data report (to two decimal places) is used to calculate the associated measurement uncertainty (with coverage factor k=3, 99.7% confidence level).

a. Example: THC is measured at 7.85 ng/mL.

b. Multiply the full result of 7.85 ng/mL by 0.26 (26%), to obtain an uncertainty of 2.041 ng/mL.

c. The THC result is truncated to 7.8 ng/mL, and the associated uncertainty is rounded to 2.0 ng/mL (both two significant figures) for reporting.

NOTE: When inputting THC results in LIMS, the full result from the data report (to two decimal places) is entered (LIMS calculates the associated uncertainty from this full result). The final THC result and associated uncertainty that appear on the final test report are verified independently (at time of issue and at time of technical review), as described in the example in 27.12.1.1, above.

27.12.2 THCCOOH results are reported to two significant figures, in units of ng/mL. Measurement uncertainty for THCCOOH is not included on the test report, but is available upon customer request.

a. Example: THCCOOH is measured at 122.52 ng/mL.

b. The THCCOOH result is truncated to 122 ng/mL (three significant figures), but reported as 120 ng/mL (two significant figures).

27.12.3 Additional information on measurement uncertainty is found in the document *Estimation and Reporting of Measurement Uncertainty, PQ12706.*

27.12.4 When multiple dilutions are analyzed, the smallest dilution within the dynamic range is reported.

27.12.5 When confirmed using this assay, urine results are reported qualitatively.

27.13 METHOD PERFORMANCE

27.13.1	Limit of detection:	THC	0.5 ng/mL
		THCCOOH	2.5 ng/mL
27.13.2	Lower limit of quantification:	THC	1.0 ng/mL
		THCCOOH	5.0 ng/mL

27.13.3	Dynamic range:	THC	1.0 – 100 ng/mL
		THCCOOH	5.0 – 500 ng/mL
27.13.4	Upper limit of quantification:	THC	100 ng/mL
		THCCOOH	500 ng/mL

27.14 TRACEABILITY

27.14.1 Traceability of the reference materials to SI units is provided through the certificate of analysis provided by the approved reference material supplier.

27.15 REFERENCES

27.15.1 A. Black, B.E. O'Reilly, in-house development.

27.15.2 D.M. Schwope, K.B. Scheidweiler, M.A. Huestis. Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography – tandem mass spectrometry. *Analytical Bioanalytical Chemistry*. 401(4):1273-1283 (2011).

27.15.3 J. Hudson, J. Hutchings, C. Harper, R. Wagner, Validation of a Cannabinoid Quantitation Method Using an Agilent 6430 LC/MS/MS, *Agilent Application Note 5991-2554EM*, June 2013.

27.15.4 Pat Friel, Agilent Technologies, Inc.

27.15.5 Virginia Department of Forensic Sciences, Cannabinoid Quantitation/Confirmation method.

APPENDIX A
INSTRUMENTAL PARAMETERS

Agilent LC-MSMS System

LIQUID CHROMATOGRAPH

Gradient Elution	
Flow Rate	0.5 mL/min
Solvent A	0.1% Formic acid
Solvent B	ACN
Initial Composition	60% A, 40% B
Hold time	1 min (40% B)
1-7 min	% B increased to 95%
Hold time	3 min (95% B)
10-10.5 min	% B decreased to 40%
Re-equilibration	2.0 minutes
Column Temp	50°C
Autosampler	
Injection Volume	10.0 µL
Injection flush-port	Active
Flush-port time	5 sec
Flush-port solvent	75:25 MeOH:DI H_2O

MASS SPECTROMETER

Ion mode	(+) MRM	Nebulizer gas	Nitrogen
Peak width	0.05 min	Nebulizer pressure	40 psi
Dwell time (Time Segment 2)	50 msec	Drying gas	Nitrogen
Dwell time (Time Segment 3)	100 msec	Drying gas flow	10.0 L/min
Time segment 1 (Time 0 min)	To Waste	Drying gas temp	350°C
Time segment 2 (Time 4.0 min)	To MS (EMV +400)		
Time segment 3 (Time 6.8 min)	To MS (EMV +400)		
Time segment 4 (Time 8.5 min)	To Waste		

Signals	MRM Transitions
THCCOOH-d_3	348.2→330.2, 302.2
THCCOOH	345.2→299.2, 193.1
THC-d_3	318.2→196.1, 123.0
THC	315.2→193.1, 123.0

Shimadzu/Sciex LC-MSMS System

SHIMADZU LIQUID CHROMATOGRAPH

Gradient Elution	
Flow rate	0.5 mL/min
Solvent A	0.1% Formic acid
Solvent B	ACN
Initial composition	60% A, 40% B
0 – 1.0 min	40% B
1.0 – 7.0 min	95% B
7.0 – 10.0 min	95% B
10.1 – 12.5 min	40% B
Post time	2.5 min
Column temp	50°C
Autosampler	
Injection volume	10 µL
Rinsing volume	1000 µL
Rinsing solvent	75:25 MeOH:DI H_2O
Cooler temperature	25ºC

SCIEX MASS SPECTROMETER

Scan type	(+) MRM	Curtain/collision gas	Nitrogen
Ion mode	ESI	Curtain gas flow	40 L/min
Resolution (Q1)	Unit	Collision gas flow	4 L/min
Resolution (Q3)	Unit	Gas 1 temp	40ºC
Valve position A (Time 0 min)	To waste	Gas 2 temp	80ºC
Valve position B (Time 1.5 min)	To MS	Ion voltage	5.5 kV
Valve position A (Time 7.5 min)	To waste	Interface temp	650ºC

Compound	MRM Transitions	Dwell Time
THCCOOH-d_3	348.3→330.0, 302.0	50 msec
THCCOOH	345.4→299.2, 193.3	50 msec
THC-d_3	318.3→196.3, 123.1	100 msec
THC	315.2→193.3, 123.2	100 msec

LIST OF CHANGES

Revision Date	Description	Page Number
5/8/14	Method approved by Washington State Toxicologist. See DRA dated 05/5/14. Method released for use in evidentiary testing on 5/8/14.	All
12/5/14	Instrumental parameters for liquid chromatograph updated to reflect an increase in the temperature of the analytical column from 40ºC to 50ºC. See DRA dated 12/5/14.	11
10/7/15	Edited 27.1 for deviation approval by a member of TLD Management. Added 27.10.1.4 to indicate that no points may be dropped from the THC calibration curve in order for the batch to be acceptable for reporting THC. Edited 27.8 for use of either the Agilent or the Sciex instruments/methods and added MultiQuant to 27.9.1 for batch data analysis. Included Shimadzu/Sciex LCMSMS instrument parameters in Appendix A. See DRA dated 9/30/15.	1, 7-8, 12
4/6/16	Added clarification to 27.6.3.2.c for use of same CRM in preparation of working standard and working control standard. Added note regarding CRM or stock standard expiration dates in 27.6.1.3, 27.6.1.4 and 27.6.1.5. Added instructions for calculation of THC measurement uncertainty, including the example and note in 27.12.1.1. Other minor edits throughout.	4, 9-10
7/24/17	Wording added to 27.4.3 regarding dilution and standard volume testing. Preparation of the control working standard was added to section 27.6.3.2. Specified use of calibrated pipettes for measurement of blank blood, specimens, and standards throughout section 27.7 SAMPLE PREPARATION. Edited 27.10.10.2.e to indicate all positive controls must meet acceptability criteria to report quantitative results. Other minor edits throughout.	1-10